

# Cytidine-5'-diphosphocholine (Citicoline): a pilot study in patients with non-arteritic ischaemic optic neuropathy

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**Background and purpose:** Our work evaluates visual function before and after treatment with cytidine-5-diphosphocholine (Citicoline) in patients with non-arteritic ischaemic optic neuropathy (NION). **Methods:** Twenty-six patients in which at least 6 months elapsed from NION, were randomly divided into two age-similar groups: 14 patients had Citicoline (Cebrolux-Tubilux, Italy, 1600 mg/diem for 60 days, followed by a 120-day period of wash out, days 60–180) (T-NION); 12 patients had no treatment during the same period (NT-NION). At day 180, in T-NION a second period of treatment (days 181–240) followed by a wash-out (days 241–360) was performed. Fourteen age-matched healthy subjects provided normative data. In all patients, pattern-electroretinogram (PERG), visual evoked potentials (VEPs) and visual acuity (VA) measurements were performed at baseline and at days 60 and 180. In T-NION, further measurements were achieved at days 240 and 360. **Results:** At baseline, NT-NION and T-NION patients showed abnormal PERGs and VEPs, and reduced VA, compared to controls. At the end of treatment (days 60 and 240), T-NION patients showed improvement ( $P < 0.01$ ) of PERGs, VEPs parameters and VA, compared to pre-treatment values. After wash out, functional improvements persisted compared to baseline. No changes in NT-NION patients were observed. **Conclusions:** Our results suggest a beneficial effect of oral Citicoline in NION.

## Introduction

Non-arteritic anterior ischaemic optic neuropathy (NION) is an irreversible ischaemic event of the intra-ocular optic nerve, occurring suddenly and usually painlessly, that induces a loss in visual acuity (VA) and visual field [1]. An average of 10% of NION patients report ocular pain or headache as ancillary symptoms [2]. Even if NION typically occurs after the age of 50 [3,4], but it has been described also in younger patients [5]. NION usually tends to be unilateral, although subjects affected bilaterally were also observed after surgical procedures [6,7].

In order to induce a stabilization or an improvement of visual function in patients affected by NION, several pharmacological options have been tested, such as systemic corticosteroids, anticoagulants, hyperbaric oxygen, and diphenylhydantoin, but none of them with proved efficacy [8]. Despite some reported improvement

of VA in NION after the use of levodopa and carbidopa [9], this observation was not confirmed by others [10]. Optic nerve decompression has been almost abandoned, because it was not supported by the findings of a trial [11].

It is noteworthy that neuroprotective strategies have not been systematically studied. The few attempted treatments failed to show any encouraging effect in NION [12,13]. Amongst various neuroprotective substances, the citicoline (cytidine-5'-diphosphocholine) is a candidate drug. Previous studies support its favourable effect in many CNS diseases characterized by hypoxia and ischaemia [14,15] such as stroke [16], brain trauma [17], Parkinson's [18] and Alzheimer's diseases [19]. The drug efficacy seems to be related to its ability to stimulate some brain neurotransmitter systems, including the dopaminergic one, known to be largely expressed in both retina and post-retinal visual pathways [20]. Clinical studies reported benefits of citicoline treatment also in optic nerve degenerative disease such as open angle glaucoma (OAG), in which it was observed that 75% of OAG patients showed an improved perimetric condition after both short and long-term parenteral (intramuscular) administration [21,22]. Even though perimetric analysis gives a psychophysical assessment of visual function, it has been observed that citicoline increases the level of consciousness [23–27]

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and, thus it is unclear whether the observed changes in glaucomatous visual field [22] could be ascribed to a better performance during visual field examination, to therapeutic effects on impaired retinal and post-retinal visual structures, or both.

By using simultaneous recordings of pattern electroretinogram (PERG), which represents an objective method to evaluate the functional integrity of the innermost retinal layers [28], and visual evoked potentials (VEPs), which reflect the bioelectrical activity of the visual cortex in response to visual stimuli [29] it was observed that, in glaucoma patients [30,31] citicoline treatment could induce an enhancement of retinal function and a reduction of the delay in post-retinal neural conduction, resulting in an improvement of visual cortical responses. It is known that both glaucoma [32] and NION [33] share similar pathophysiological mechanisms, amongst others an ischaemic factor that is able to produce RGCs apoptosis [34].

Taking into account the latter evidence, in our study we investigated retinal function and neural conduction in the visual pathways of patients affected by NION, using simultaneous recording of VEPs and PERGs. We also assessed whether oral treatment with citicoline may induce any affect on retinal and visual pathways function. In this study, the effects of the drug on visual field sensitivity were purposefully not considered, since any detectable improvement could be ascribed to the associated effects on consciousness level and attention [23–27].

## Methods

### Patients

Twenty-six volunteer patients affected in only one eye by NION (mean age  $65.4 \pm 3.8$  years) and 14 age-matched control subjects (mean age  $63.8 \pm 4.2$  years) took part in the study.

In all NION patients enrolled, the diagnosis of non-arteritic ischaemic optic neuropathy was made on the basis of the following criteria [8,35]: (i) occurring after the age of 50 years, (ii) at least 6 months after an episode of sudden, painless, unilateral visual loss accompanied by an afferent pupil defect and/or acquired dichromatopsia, and a normal fellow eye with no evidence of any optic nerve or ocular disease, (iii) visual field defects on Goldman perimetry such as altitudinal (inferior or superior) scotoma that, in some cases, reached, but not completely, the central visual field and (iv) normal intraocular pressure measurement and no abnormalities of the anterior segment in both eyes at slit lamp examination.

Exclusion criteria for arteritic anterior ischaemic optic neuropathy were signs, symptoms or laboratory findings suggesting giant cell arteritis: age  $> 70$  years, headache, abnormal temporal artery on physical examination, fever (temperature  $\geq 38^\circ\text{C}$ ), jaw claudication, high inflammatory response, as higher values of erythrocyte sedimentation rate (ESR), CRP C-reactive protein (CRP) and platelet counts, and high immunoglobulin serum levels (high  $\alpha$ -2 globulin) [36].

For both NION patients and control subjects, exclusion criteria were: presence of moderate to dense lens opacities or maculopathy which are known to affect the PERG [37,38], implanted intraocular lens, presence of corneal opacities, previous history of refractive surgery, glaucoma or ocular hypertension, intraocular inflammation such as anterior or posterior uveitis, retinal detachment or laser treatment for peripheral retinal diseases, ocular trauma, diabetes and other systemic or neurological diseases.

### Pharmacological treatment and testing schedule

The 26 enrolled NION patients were randomly (see below) divided into two age-similar groups: in 14 patients a pharmacological treatment with a daily oral dose of 1600 mg citicoline (Cebrolux<sup>®</sup>, Tubilux, Pomezia, Italy) was performed over 60 days (0–60 days: first period of pharmacological treatment with citicoline), followed by a 60-days period of wash out (days 1–180) (Treated NION, T-NION, mean age  $64.6 \pm 3.3$  years, 14 NION eyes). Twelve NION patients were only observed without any pharmacological treatment (non-treated NION, NT-NION; mean age:  $66.2 \pm 4.2$ ; 12 eyes)

When in T-NION we observed a worsening trend of electrophysiological parameters (see below: results at day 180), we decided to provide in these patients a second 60-day period of pharmacological treatment with citicoline followed by a second period of wash-out (241–360 days); the follow-up was at 360 days.

Informed consent was obtained from each patient enrolled in this study and the research followed the tenets of the Declaration of Helsinki. The study was previously approved by the local Ethical Committee.

### Electrophysiological examinations

In both control subjects and in NION patients, simultaneous PERG and VEP recordings were performed at baseline. Then, follow-up recordings were obtained in NION patients at 60, 120, 240 and 360 days, according to the above reported testing schedule.

Simultaneous recording of PERGs and VEPs using high (80%) contrast 15' checkerboard stimuli reversed at the rate of two reversals per second were obtained following a previously published protocol [39–41].

For all VEPs and PERGs the implicit time and the peak amplitude of each of the waves were measured directly on the displayed records by means of a pair of cursors. Simultaneous recordings of VEPs and PERGs allow us to derive the retinocortical time (RCT) as the difference between the VEP P100 and the PERG P50 peak latencies [37].

At baseline, all control and NION eyes underwent at least two simultaneous recording of PERG and VEP, 1–7 days apart, to determine test–retest variability. During the follow-up assessment (60, 180, 240 and 360 days) PERG and VEP were performed in NION patients at least three times and the resulting waveforms were superimposed to check the repeatability of the results. The recording with the highest PERG P50–N95 amplitude was considered in the statistical analysis (see below). During all follow-up examinations, PERG and VEP recordings were performed in a condition of pupil diameter equal to that measured in baseline conditions (see above).

In order to evaluate the presence of normal or abnormal PERG and VEP responses, independently from the clinical conditions of the tested subjects, all electrophysiological examinations were performed at baseline conditions in the presence of two operators (GC and GG), who did not know if the tested subject belonged to the category of control subjects or NION patients (treated or untreated), as classified by two other operators (VP and BF). The random separation in treated and untreated patients was performed, in accordance with an electronically generated randomization table, by one operator (LZ) who was the only one to know the key. Indeed, during all electrophysiological recordings performed in NION patients at 60 and 180 days of follow-up, GC and GG were unable to know whether the tested patient belonged to the treated or untreated group. The key was opened only at the end of the first wash-out period (day 180). Since it was decided to perform a further period of treatment in T-NION eyes, (see above), the electrophysiological assessment carried out in these patients at day 240 and 240 were performed knowing that the tested patients belonged to treated group.

### Best-corrected visual acuity

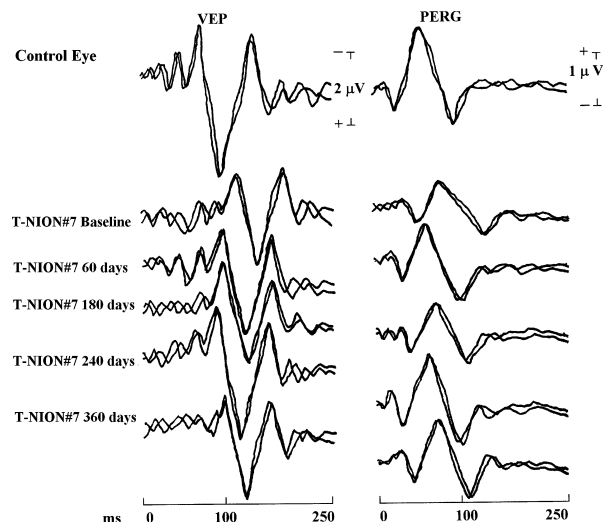
Best-corrected VA, assessed by VP or BF, was evaluated by the modified Early Treatment Diabetic Retinopathy Study (ETDRS) Table (Lighthouse, Low vision products, Long Island City, NY, USA) at the

distance of 4, 2 and 1 min. VA was measured in logarithm of minimum angle of resolution (LogMAR) values and in equivalent Snellen's values (from 20/200 = 0.1 to 20/20 = 1.0).

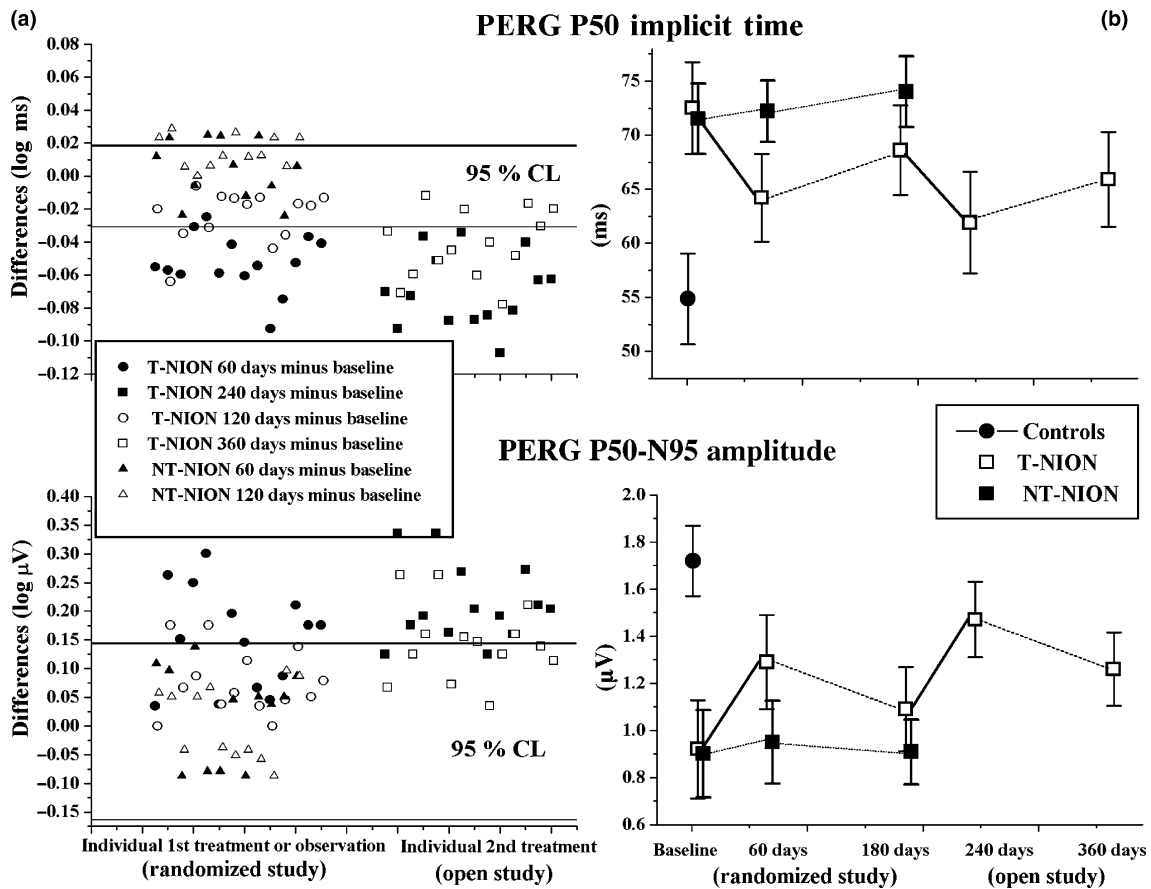
### Statistics

The STATISTICA (data analysis software system) for Windows, version 7, was used for all analyses [StatSoft Inc., 2004, (Tulsa, OK, USA) <http://www.statsoft.com>].

Sample size estimates were obtained from pilot evaluations performed in 20 eyes from 20 NION patients and 14 eyes from 14 control subjects, other than those included in the current study (V. Parisi, G. Coppola, L. Ziccardi, unpublished data). Inter-individual variability, expressed as data standard deviation (SD) was estimated for PERG P50–N95 amplitude and VEP P100 implicit time measurements. It was found that data SDs were significantly greater for patients when compared to controls (about 35% vs. 15%). It was also established that, assuming the above between-subjects SD in the current study, sample sizes of control subjects and patients belonging to NION group provided a power of



**Figure 1** Layout of simultaneous VEP and PERG recordings in a control subject and in one patient with non-arteritic ischaemic optic neuropathy treated with citicoline (T-NION7). Electrophysiological recordings were collected in baseline condition and 60, 180, 240 and 360 days after citicoline treatment. Treatment was administered in two different 60-day periods (0–60 and 181–240 days) followed by two periods of wash-out (61–180 and 241–360 days). In comparison with the baseline condition, in T-NION#7 patient, the VEP and PERG recorded after citicoline treatment showed a decrease in implicit time and an increase in amplitude. Nevertheless, VEP and PERG implicit times and amplitudes were still increased and reduced with respect to those from the control eye.



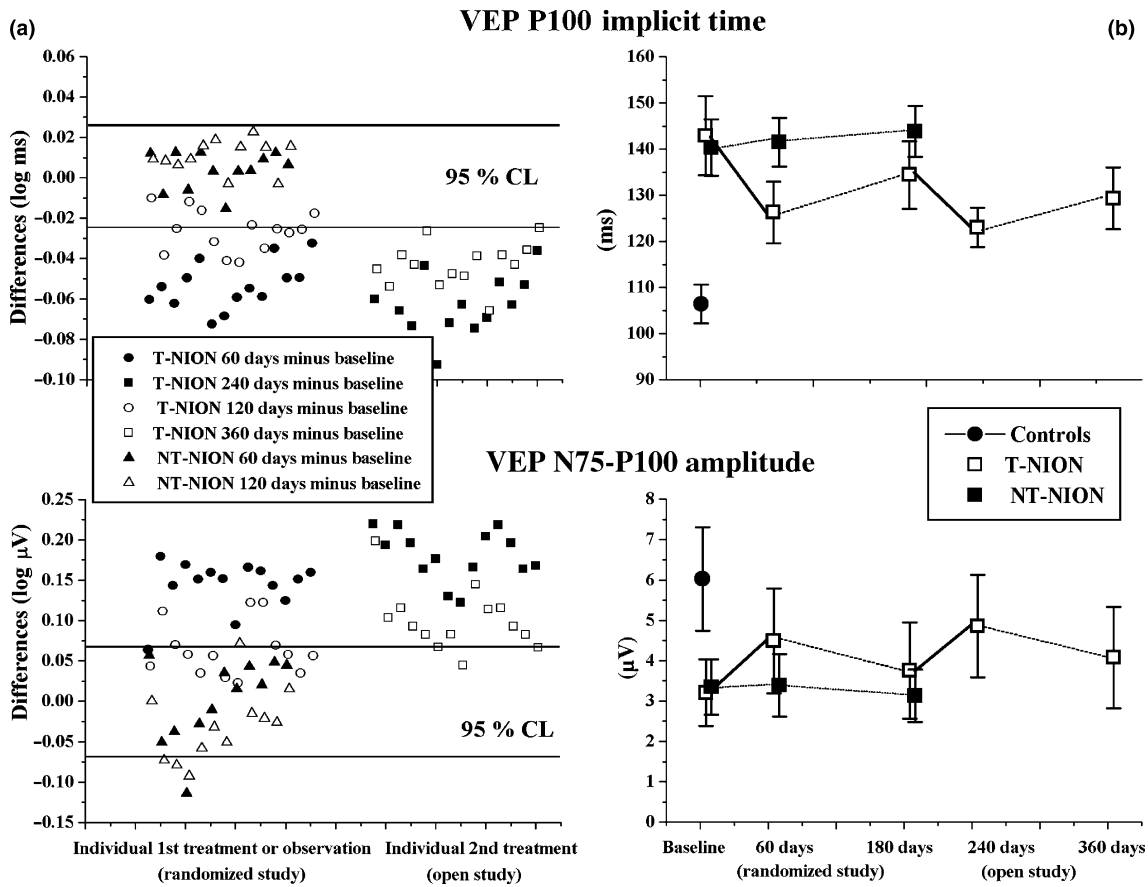
**Figure 2** (a) Individual changes in pattern electroretinogram (PERG) responses observed in eyes affected by non-arteritic ischaemic optic neuropathy untreated (NT-NION) or treated with citicoline (T-NION). Values refer to the difference between values observed after 60, 180, 240 and 360 days with respect to their baseline condition. In T-NION eyes, the medical treatment with citicoline was performed over a 60-day period followed by 120 days of wash-out. At day 180, a second 60-day period of citicoline treatment followed by a second period of 180 days of wash-out were performed. Solid and dashed lines refer respectively to the upper and lower 95% confidence limit of the intra-individual variability resulting from test–retest analysis. (b) Graphic representation of mean values of PERG P50 implicit time and P50-N95 amplitude observed in control subjects and in patients affected by NION in baseline condition and after medical treatment with citicoline (T-NION) or observation (NT-NION). The solid lines indicate the periods of medical treatment, whilst the dashed lines indicate the periods of wash-out. Vertical lines represent one standard deviation of the mean. The statistical analysis evaluating the differences between controls, T-NION and NT-NION groups and within T-NION group are reported in Tables 1 and 2.

80%, at an  $\alpha = 0.05$ , for detecting a between-group difference of 55% in PERG P50-N95 amplitude and 25% VEP P100 implicit time measurements. These differences were preliminarily observed by comparing NION and control data (see above). A sample size of 20 patients and 14 control subjects also allowed a detection of average test–retest differences in PERG amplitude of 25% (with an SD of residuals of 20%), and in VEP implicit time of 20% (with an SD of residuals of 20%). These changes were expected to be clinically meaningful when comparing results of treated NION eyes observed in baseline conditions versus those observed at 60, 180, 240 and 360 days.

Test–retest data of PERG and VEP results were expressed as the mean difference between two

recordings obtained in separate sessions  $\pm$  the SD of this difference. About 95% confidence limits of test–retest variability in normal subjects and patients were established assuming a normal distribution.

The differences of PERG and VEP responses and Visual Acuity values between groups (Control eyes, NT-NION and T-NION eyes) were evaluated by analysis of variance (ANOVA). ANOVA for repeated measurements was used for the analysis of each single considered parameter (PERG, VEP and VA) in NT-NION and T-NION patients versus baseline measurements. A two-way ANOVA, considering group (NT-NION, T-NION) as between subjects factor and time points (baseline: 60–



**Figure 3** (a) Individual changes in visual evoked potential (VEP) responses observed in eyes affected by non-arteritic ischaemic optic neuropathy untreated (NT-NION) or treated with citicoline (T-NION). Values refer to the difference between values observed after 60, 180, 240 and 360 days with respect to their baseline condition. Solid and dashed lines refer respectively to the upper and lower 95% confidence limit of the intra-individual variability resulting from test-retest analysis. (b) Graphic representation of mean values of VEP P100 implicit time and N75-P100 amplitude observed in control subjects and in patients affected by NION in baseline condition and after medical treatment with citicoline (T-NION) or observation (NT-NION). The solid lines indicate the periods of medical treatment, whilst the dashed lines indicate the periods of wash-out. Vertical lines represent one standard deviation of the mean. The statistical analysis evaluating the differences between controls, T-NION and NT-NION groups and within T-NION group are reported in Tables 1 and 2.

180 days) as within subject factors was employed to compare changes over time in PERG and VEP parameters recorded in NT-NION and T-NION patients.

In all analyses, a conservative *P*-value less than 0.01, to compensate for multiple comparisons, was considered as statistically significant.

**Results**

Examples of simultaneous recordings of VEP and PERG performed in one eye from one Control subject and in one eye from one T-NION patient before and after the medical treatment with citicoline are displayed in Fig. 1.

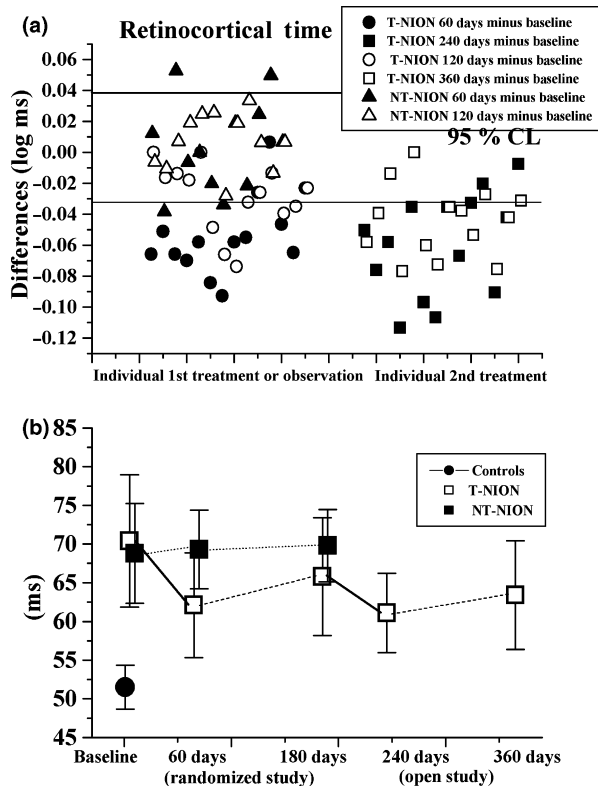
The individual changes observed in NION eyes at 60 and 180 days (in NT-NION and T-NION eyes) and at

240 and 360 days (in T-NION eyes) of follow-up with respect to baseline conditions are shown in Figs 2a and 3a.

The mean data and the statistical analysis are presented in Figs 2b, 3b, 4 and 5, and in Tables 1 and 2.

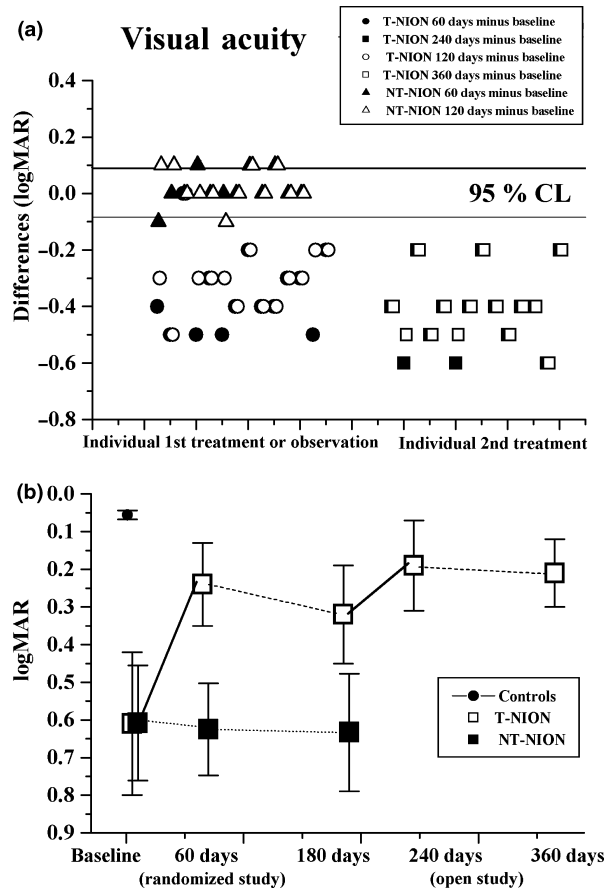
In baseline condition, both NT-NION and T-NION eyes showed PERG P50 and VEP P100 implicit times and RCT significantly (*P* < 0.01) delayed, P50-N95 amplitude significantly (*P* < 0.01) reduced, VA significantly (*P* < 0.01) reduced, when compared to those observed in controls.

After 60 days of citicoline treatment (day 60), T-NION eyes showed an increase in VA, a shortening in PERG P50 and VEP P100 implicit times and in RCT and an increase in PERG P50-N95 and VEP N75-P100 amplitudes (*P* < 0.01) with respect to the baseline values. After 120 days of wash-out (day 180),



**Figure 4** (a) Individual changes in retinocortical time (difference between VEP P100 and PERG P50 implicit times) observed in eyes affected by non-arteritic ischaemic optic neuropathy untreated (NT-NION) or treated with citicoline (T-NION). Values refer to the difference between values observed after 60, 180, 240 and 360 days with respect to their baseline condition. Solid and dashed lines refer respectively to the upper and lower 95% confidence limit of the intra-individual variability resulting from test–retest analysis. (b) Graphic representation of mean values of retinocortical time observed in control subjects and in patients affected by NION in baseline condition and after medical treatment with citicoline (T-NION) or observation (NT-NION). The solid lines indicate the periods of medical treatment, whilst the dashed lines indicate the periods of wash-out. Vertical lines represent one standard deviation of the mean. The statistical analysis evaluating the differences between controls, T-NION and NT-NION groups and within T-NION group are reported in Tables 1 and 2.

T-NION eyes showed a reduction in VA, an increase in PERG P50 and VEP P100 implicit times and in RCT and a decrease in PERG P50-N95 and VEP N-75-P100 amplitudes with respect to the values observed at 60 days. Nevertheless, VA, PERG P50 and VEP P100 implicit times and RCT and the PERG P50-N95 and VEP N-75-P100 amplitudes were still shorter and increased, but not significantly different ( $P > 0.1$ ), than those observed in baseline condition. In the same period non-significant ( $P > 0.01$ ) VA, PERG and VEP changes were detected in NT-NION eyes.



**Figure 5** (a) Individual changes in logMAR visual acuity (VA) observed in eyes affected by non-arteritic ischaemic optic neuropathy untreated (NT-NION) or treated with citicoline (T-NION). Values refer to the difference between values observed after 60, 180, 240 and 360 days with respect to their baseline condition. Solid and dashed lines refer respectively to the upper and lower 95% confidence limit of the intra-individual variability resulting from test–retest analysis. (b) Graphic representation of mean values of logMAR VA observed in control subjects and in patients affected by NION in baseline condition and after medical treatment with citicoline (T-NION) or observation (NT-NION). The solid lines indicate the periods of medical treatment, whilst the dashed lines indicate the periods of wash-out. Vertical lines represent one standard deviation of the mean. The statistical analysis evaluating the differences between controls, T-NION and NT-NION groups and within T-NION group are reported in Tables 1 and 2.

After the second period of 60 days of citicoline treatment (day 240), in T-NION eyes a further increase in VA together with a shortening in PERG P50 and VEP P100 implicit times and in RCT and a further increase in PERG P50-N95 and VEP N-75-P100 amplitudes ( $P < 0.01$ ) with respect to the values observed at 180 days were found. At day 360, after a second period of 120 days of wash-out, a decrease in

**Table 1** Statistical evaluation (one-way analysis of variance, ANOVA) between citicoline-treated non-arteritic ischaemic optic neuropathy patients group (T-NION) and control eyes group and within T-NION group

	ANOVA		
	T-NION vs. controls	T-NION vs. baseline	T-NION vs. previous
<b>PERG P50 implicit time</b>			
Baseline	$F(1,25): 79.47, P < 0.01$		
60 days (first treatment)	$F(1,25): 23.13, P < 0.01$	$F(1,27): 27.90, P < 0.01$	
180 days (first follow-up)	$F(1,25): 49.14, P < 0.001$	$F(1,27): 6.09, P = 0.020$	$F(1,27): 7.89, P = 0.009$
240 days (second treatment)	$F(1,25): 11.81, P < 0.01$	$F(1,27): 39.3, P < 0.01$	$F(1,27): 15.90, P = 0.0005$
360 days (second follow-up)	$F(1,25): 30.70, P < 0.01$	$F(1,27): 16.6, P < 0.01$	$F(1,27): 5.42, P = 0.028$
<b>PERG P50-N95 amplitude</b>			
Baseline	$F(1,25): 136.2, P < 0.01$		
60 days (first treatment)	$F(1,25): 41.42, P < 0.01$	$F(1,27): 22.6, P < 0.01$	
180 days (first follow-up)	$F(1,25): 101.2, P < 0.001$	$F(1,27): 4.81, P = 0.037$	$F(1,27): 7.76, P = 0.0098$
240 days (second treatment)	$F(1,25): 18.19, P < 0.01$	$F(1,27): 60.4, P < 0.01$	$F(1,27): 33.6, P < 0.01$
360 days (second follow-up)	$F(1,25): 65.83, P < 0.01$	$F(1,27): 24.4, P < 0.01$	$F(1,27): 11.8, P = 0.002$
<b>VEP P100 implicit time</b>			
Baseline	$F(1,25): 203.6, P < 0.01$		
60 days (first treatment)	$F(1,25): 89.24, P < 0.01$	$F(1,27): 32.4, P < 0.01$	
180 days (first follow-up)	$F(1,25): 154.1, P < 0.001$	$F(1,27): 7.93, P < 0.01$	$F(1,27): 9.29, P < 0.01$
240 days (second treatment)	$F(1,25): 106.7, P < 0.01$	$F(1,27): 59.7, P < 0.01$	$F(1,27): 25.0, P < 0.01$
360 days (second follow-up)	$F(1,25): 117.4, P < 0.01$	$F(1,27): 21.7, P < 0.01$	$F(1,27): 8.74, P < 0.01$
<b>VEP N75-P100 amplitude</b>			
Baseline	$F(1,25): 48.18, P < 0.01$		
60 days (first treatment)	$F(1,25): 9.98, P < 0.01$	$F(1,27): 9.26, P < 0.01$	
180 days (first follow-up)	$F(1,25): 23.62, P < 0.001$	$F(1,27): 1.89, P = 0.181$	$F(1,27): 2.44, P = 0.120$
240 days (second treatment)	$F(1,25): 5.89, P = 0.02$	$F(1,27): 15.7, P < 0.01$	$F(1,27): 5.59, P = 0.025$
360 days (second follow-up)	$F(1,25): 16.50, P < 0.01$	$F(1,27): 4.42, P = 0.045$	$F(1,27): 2.64, P = 0.116$
<b>Retinocortical time</b>			
Baseline	$F(1,25): 310.0, P < 0.01$		
60 days (first treatment)	$F(1,25): 29.04, P < 0.01$	$F(1,27): 8.06, P < 0.01$	
180 days (first follow-up)	$F(1,25): 43.39, P < 0.001$	$F(1,27): 2.23, P = 0.147$	$F(1,27): 1.86, P = 0.184$
240 days (second treatment)	$F(1,25): 37.53, P < 0.01$	$F(1,27): 12.0, P < 0.01$	$F(1,27): 3.59, P = 0.069$
360 days (second follow-up)	$F(1,25): 34.66, P < 0.01$	$F(1,27): 5.49, P = 0.027$	$F(1,27): 0.963, P = 0.334$
<b>logMAR visual acuity</b>			
Baseline	$F(1,27): 118.5, P < 0.01$		
60 days (first treatment)	$F(1,27): 38.03, P < 0.01$	$F(1,27): 37.76, P < 0.01$	
180 days (first follow-up)	$F(1,27): 57.29, P < 0.001$	$F(1,27): 22.22, P < 0.01$	$F(1,27): 3.09, P = 0.091$
240 days (second treatment)	$F(1,27): 17.28, P < 0.01$	$F(1,27): 48.90, P < 0.01$	$F(1,27): 7.56, P = 0.011$
360 days (second follow-up)	$F(1,27): 40.27, P < 0.01$	$F(1,27): 50.68, P < 0.01$	$F(1,27): 0.25, P = 0.622$

**Table 2** Statistical evaluation (ANOVA for repeated measures) between not treated non-arteritic ischaemic optic neuropathy patients group (NT-NION) and citicoline-treated non-arteritic ischaemic optic neuropathy patients group (T-NION). Between subjects factor, group (NT-NION, T-NION), within subjects factor, time points (baseline – 60 day – 180 days), and their interactions (time × group)

	Between subjects factor: group (NT-NION, T-NION)	Within subjects factor: time (baseline, 60 days, 180 days)	
			Interactions group by time
<b>PERG</b>			
P50 implicit time (ms)	$F(2,75): 9.51, P = 0.005$	$F(2,75): 30.87, P < 0.001$	$F(2,75): 40.10, P < 0.001$
P50-N95 amplitude ( $\mu$ V)	$F(2,75): 7.94, P = 0.009$	$F(2,75): 24.02, P < 0.001$	$F(2,75): 13.83, P < 0.001$
<b>VEP</b>			
P100 implicit time (ms)	$F(2,75): 8.43, P = 0.007$	$F(2,75): 71.02, P < 0.001$	$F(2,75): 94.21, P < 0.001$
N75-P100 amplitude ( $\mu$ V)	$F(2,75): 2.09, P = 0.16$	$F(2,75): 41.10, P < 0.001$	$F(2,75): 33.82, P < 0.001$
Retinocortical time (ms)	$F(2,75): 1.72, P = 0.20$	$F(2,75): 10.98, P < 0.001$	$F(2,75): 14.28, P < 0.001$
Visual acuity: logMAR	$F(2,75): 20.5, P < 0.001$	$F(2,75): 38.85, P < 0.001$	$F(2,75): 49.38, P < 0.001$
Snellen	$F(2,75): 19.8, P < 0.001$	$F(2,75): 36.79, P < 0.001$	$F(2,75): 42.16, P < 0.001$

VA with respect to the values observed at 240 days was found. Nevertheless, the VA values were still lower than the basal ones ( $P < 0.01$ ) and therefore, the improvement in VA was maintained. An increase in PERG P50 and VEP P100 implicit times and in RCT and a decrease in PERG P50-N95 and VEP N-75-P100 amplitudes with respect to the values observed at 240 days was found. PERG P50 and VEP P100 implicit times and in RCT and the PERG P50-N95 and VEP N-75-P100 amplitudes were still significantly ( $P < 0.01$ ) shorter and increased with respect to baseline ones.

During the entire period of the study (0–360 days), notwithstanding the changes observed in T-NION eyes after the treatment, all electrophysiological (PERG P50 implicit times and P50-N95 amplitude, VEP P100 implicit time and N75-P100 amplitude, RCT) or psychophysical (logMAR VA) parameters were always significantly ( $P < 0.01$ ) different from those of controls.

During the whole period of treatment, no adverse side effects were reported from any of the patients enrolled in the study.

## Discussion

Our study was designed to evaluate the retinal and visual cortical responses in patients affected by NION using simultaneous recordings of VEPs and PERGs before and after treatment with oral citicoline.

Treatment with citicoline induced an electrophysiological improvement involving both retinal responses (PERGs increase in amplitudes and shortening in implicit time), and post-retinal visual responses (VEPs increase in amplitudes and shortening in both implicit time and RCT). No such effects were found in the untreated patients. Unlike the relatively constant electro-functional improvement, citicoline induced the maximum increase of VA only after the first period of treatment (0–60 days). In fact, after this initial steep increase, we observed only a little progression of the VA at the end of the second treatment phase (180–240 days). Testing VA may not be fully dependable, because the measured value is highly subjective and influenced by the suggested citicoline effects of increased level of consciousness and attention [23–38,42].

Although our results support a favourable effect of citicoline in reducing the retinal and post-retinal NION dysfunction, the mechanism of action of citicoline in the visual system is not entirely understood. The possible mechanisms of action have been widely discussed in our previous report [30,31]. Briefly, citicoline is an endogenous mononucleotide involved as an essential intermediate in the synthesis of structural phospholipids of cell membranes, such as phosphatidylcholine [14,15,43,44].

Citicoline increases the metabolism of cerebral structures [14] and inhibits phospholipid degradation [15]. Therefore, it may have potential neuroprotective and neuromodulator roles as demonstrated in conditions of cerebral hypoxia and ischaemia [14,15]. Citicoline is known to increase in some brain areas the levels and enhance the rate of synthesis of acetylcholine, dopamine, noradrenalin, and serotonin [14,15]. Evidence coming from animal models has shown that citicoline reinforces the dopaminergic transmission in the retina [45]. It is well known that the dopaminergic system is expressed at high level in retina and post-retinal visual pathways [27], and that it acts both within synapses and by diffusion to more distant targets [46]. Certain ganglion cells sub-types use dopamine to communicate with visual cortex [47]. This is why a deficit of this neurotransmitter/neuromodulator could produce an impairment of both PERG and VEPs responses [48]. According to our results, the improvement of retinal function and neural conduction after citicoline treatment may be ascribed to a neurotransmission enhancement at both visual levels. The dopaminergic pathway indeed seems to be the ideal candidate target for a modulatory role both on peripheral (i.e. retinal ganglion cells) [49–51] and central (i.e. occipital areas) visual systems [52,53]. On the other hand, it cannot be excluded that the improvement of visual electrophysiological responses following citicoline treatment is a consequence of an overall improvement in visual fixation stability. However, PERG and VEP improvement steadily progressed throughout the treatment period whereas over the same period acuity measurements first improved and then stabilized. Therefore, it is unlikely that fixation stability had further improved after the initial steep increase in VA.

It is to be noted that all electrophysiological parameters and also the VA worsened a little after both the first (60–180 days) and the second (240–360 days) drug discontinuation, even though, compared to baseline, the improvement still persisted along the entire study period at the level of significance. We have no conclusive explanation for this phenomenon, but we can hypothesize that it could be attributed to some drug-dependence related mechanism as previously observed in glaucoma patients [30,31].

In conclusion, in our pilot study NION patients showed a dysfunction of the inner retinal layers (abnormal PERG) and an impairment of the neural conduction at post-retinal level (abnormal VEP and RCT), that could be in part improved by treatment with citicoline. Nevertheless, further study (using a more extensive cohort of patients and a placebo-treated control group) are required to confirm our preliminary results.



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